Xenogenic Vascular Grafts Implanted in a Sheep Model of Infection

Albert Grobe, PhD; Pamela Broussard, DVM; Srilaxmi Musunuri, MS; Damian Cremins, BA; Eric Wohlgehagen, RLAT; Stephen Tahta, MD and David T. Cheung, PhD
Tissue Engineering Lab and Surgical Research Lab, The International Heart Institute of Montana Foundation, Missoula, MT

Background

Extremity injuries account for 50-75% of all combat casualties with the definitive repair of vascular injuries being accomplished using autologous vessels (vein or artery). However, due to the contaminated characteristics of a wartime wound, the possibility of infection remains a concern in these patients. Based on the actual severity of the injury, infection rates can be as high as 50% for complex injuries to as low as 0%-2% for small wounds[1]. Depending on the study, gram positive (*Staphylococcus aureus)*[2] or gram negative (*Acinetobacter* spp)[3] bacteria appear to account for the majority of wound infections. When a medical device is used, the rate of device infection can be as high as 6%[4] and *Staphylococcus* species are most commonly associated with device-related infections[3].

We have developed a xenograft conduit (L+D-Hydro™) that has been treated by a two-step process resulting in a sterile, freeze-dried conduit that could be used as a vascular graft in wartime wounds. When implanted into the carotid position in sheep, this graft material exhibits superior hemodynamics and patency compared to autologous vein grafts.

The goals of this study were two-fold: To evaluate the resistance to bacterial colonization of L+D-Hydro™ xenografts (+/- antibiotic impregnation) *in vitro* as well as *in vivo* where grafts (+/- antibiotic impregnation) were compared to autologous vein grafts in an 'infected wound bed' model.

Methods

In Vitro Infectivity Model

L+D-Hydro™ vascular grafts were treated as follows: Impregnated with Rifampin (60mg/ml in sterile H_2O) for 15 minutes at 37°C, or no Rifampin treatment (Saline). The grafts were rinsed 3X in saline solution and incubated in inoculum (*Staphylococcus aureus*, 0.5 or $5x10^8$ cfu/ml) or PBS-0.25% dextrose solution for 18hrs at room temperature. All samples were rinsed with sterile PBS and sonicated on ice in PBS-dextrose for 5-10 min. The resulting liquid was collected and serial dilutions prepared. Dilutions were plated on blood agar plates and incubated for a minimum of 24 hrs at 37°C. The graft material was also homogenized in PBS-0.25% dextrose, serially-diluted, plated onto blood agar plates, and incubated for 24hrs at 37°C. Colony counts were recorded for all samples.

In Vivo Infectivity Model

L+D-Hydro™ vascular grafts were rehydrated in heparinized saline and treated as follows: Impregnated with Rifampin (60mg/ml in sterile H_2O) for 15 minutes and rinsed with sterile saline solution (+Rifampin) or soaked in sterile saline (-Rifampin). Grafts (+/- Rifampin) and autologous vein grafts were implanted as interposition grafts into the carotid position of sheep (6 each) and immediately prior to incision closure, the grafts were inoculated with 1ml of ~$1.4X10^8$ cfu/ml *S. aureus* culture. Sheep were monitored daily for signs of infection throughout the 3 week study period. At the end of 3 weeks (or sooner), the sheep were treated with heparin and the grafts were harvested with sterile technique. Grafts were opened longitudinally and photographed. The exterior and interior were lavaged with sterile tryptic soy broth and serial dilutions made of the lavage samples. Dilutions were plated onto Mannitol Salt Agar. A portion of the graft material (~0.5-1cm) was excised from the center of the sample, weighed, homogenized, sonicated on ice for 30 seconds and serial dilutions plated onto Mannitol Salt Agar. All plates were incubated for 48-72 hrs at 37°C and colony counts recorded.

In Vitro Resistance to Colonization

Expm	Inoculum (cfu/ml)	Sup (cfu/ml)	Hom (cfu/ml)	Sup (cfu/cm²)	Hom (cfu/cm²)
1-Rif	$0.5x10^8$	$2.7x10^6$	$1.37x10^5$	$1.8x10^6$	$8.99x10^4$
1+Rif	$0.5x10^8$	0	0	0	0
2-Rif	$0.49x10^8$	$8.8x10^5$	$3.09x10^4$	$5.6x10^5$	$5.19x10^4$
2+Rif	$0.49x10^8$	0	0	0	0
3-Rif	$5.3x10^8$	$9.8x10^6$	$6.36x10^5$	$6.3x10^6$	$4.09x10^5$
3+Rif	$5.3x10^8$	0	0	0	0

Table 1: Vascular grafts were incubated with Rifampin or saline for 15 minutes at 37°C, rinsed and incubated in the innoculum (*S. aureus* Xen 29/PBS-0.25% dextrose) for 18hrs at room temperature. The graft coupons were rinsed in sterile PBS to remove non-adherent bacteria, sonicated on ice and the resulting liquid collected. The remaining graft material was homogenized in buffer. Samples from the supernatant and homogenate were serially diluted, plated onto sheep blood agar plates and incubated for 24hrs at 37°C. Colony counts were recorded for the original inoculum, supernatant and homogenate. No viable bacteria were recovered from the rifampin-treated grafts while untreated grafts were readily colonized.
Experiment 1: n=2 bacteria, n=3 for rifampin/bacteria.
Experiment 2: n=5 bacteria, n=5 for rifampin/bacteria.
Experiment 3: n=5 bacteria, n=5 for rifampin/bacteria.

In Vivo Implant of Grafts

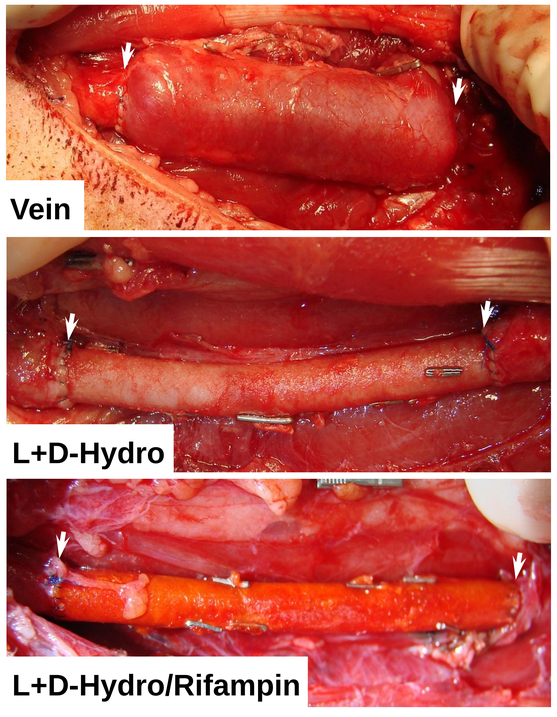

Figure 1: L+D-Hydro™grafts (treated with saline or rifampin) or autologous vein grafts were implanted into the carotid position in a sheep model. Immediately prior to closing, inoculum (*Staphylococcus aureus)* was dripped onto the surface of the graft material. The sheep were monitored for up to 3 weeks for clinical signs of infection. The anastomoses are marked by arrows. The orange color of the lower graft is due to the rifampin.

Graft Explant - Vein

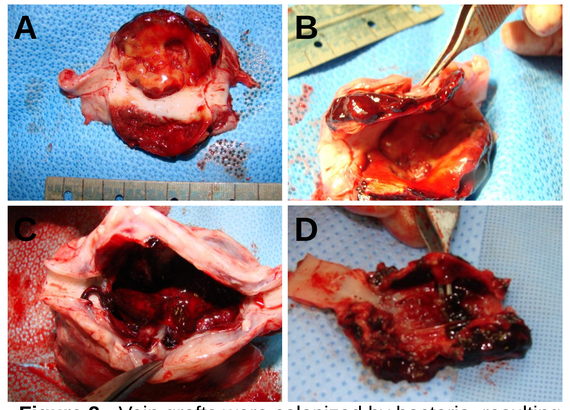

Figure 2: Vein grafts were colonized by bacteria, resulting in aneurysm (A-D), dissection (A&B) and/or thrombosis (C). In one case, the vein graft was degraded to the point of dissolution (D), resulting in death by hemorrhage. Of the 3 sheep implanted, one graft was patent at 3 weeks while two died prior to the end of the 3 week period.

Graft Explant - L+D Hydro

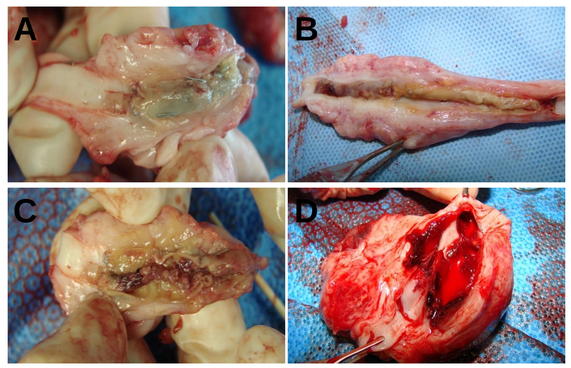

Figure 3: Untreated grafts were colonized by bacteria (A-D) leading to occlusion (B), degeneration (A-D) or hemorrhage (D). One graft was patent, two occluded, and two were patent but were sacrificed prior to 3 weeks due to hemorrhage.

Graft Explant – L+D Hydro/Rifampin

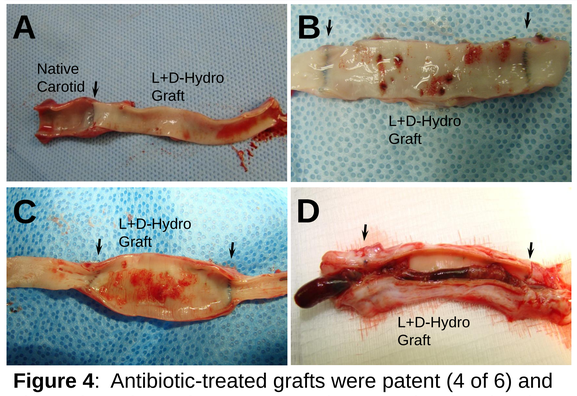

Figure 4: Antibiotic-treated grafts were patent (4 of 6) and showed no signs of aneurysm or degeneration (A-C). There were two occlusions, one of which was attributed to the procedure (D). The arrows mark the location of the anastomoses.

Treated Grafts Resistant to Colonization

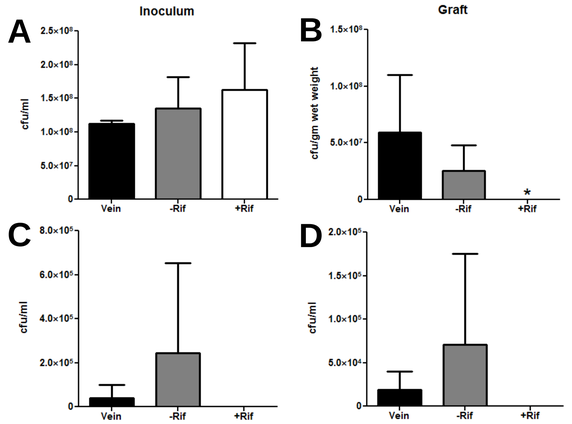

Figure 5: No statistically significant differences were seen between groups in the initial inoculum applied to grafts (A). Comparing graft types, vein grafts appeared most susceptible to bacterial colonization, followed by the L+D-Hydro™ grafts and lastly, the rifampin-treated L+D-Hydro™ grafts (B). Rifampin treatment significantly reduced the recoverable bacteria from implanted xenografts (p 0.017 Rifampin-treated vs. vein, T test). By comparison, more bacteria were recovered from lavages of L+D-Hydro™ grafts, followed by the vein and Rifampin-treated L+D-Hydro™ grafts (C&D), though the differences were not statistically significant (ANOVA). These statistics are inclusive of all collected samples without distinction for time *in vivo*.

Discussion

This study indicated that the L+D-Hydro™ xenograft material, when treated with rifampin, yielded a graft that was resistant to bacterial colonization *in vitro*. When implanted into sheep in an 'infected wound bed model', antibiotic-treated xenografts had superior patency and durability compared to autologous vein grafts and untreated xenografts. The treated xenografts were also resistant to bacterial colonization as opposed to untreated xenografts and autologous vein grafts. While further testing is necessary, these results suggest that the antibiotic-treated xenograft material may be superior to autologous vein grafts in wartime extremity vascular wounds where the potential for infection is high.

Acknowledgments

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The views, opinions and/or findings contained in this poster are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless designated by other documentation.

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